Peer Review File

Article Information: http://dx.doi.org/10.21037/tp-20-385

Reviewer Comments

This paper initially appeared to be an addition of 3 cases and an overview of an extensive number of cases it has a number of major issues which need to be rectified.

Comment 1: Not all published cases have been included - in particular it is concerning that despite the authors stating they have done an extensive literature search they have omitted a number of significant papers which include both the UK cohort of 34 patients and the Australian cohort of 18 patients.

Reply 1: We are sorry for your confusion. As described in the method section, we achieved extensive literature, including the two literatures you mentioned. (Please see the supplementary materials for detailed information of the included literatures in supplementary excel file Line 105 and Line163 in Data_original file. Figure R1 serves as a reference before you search).

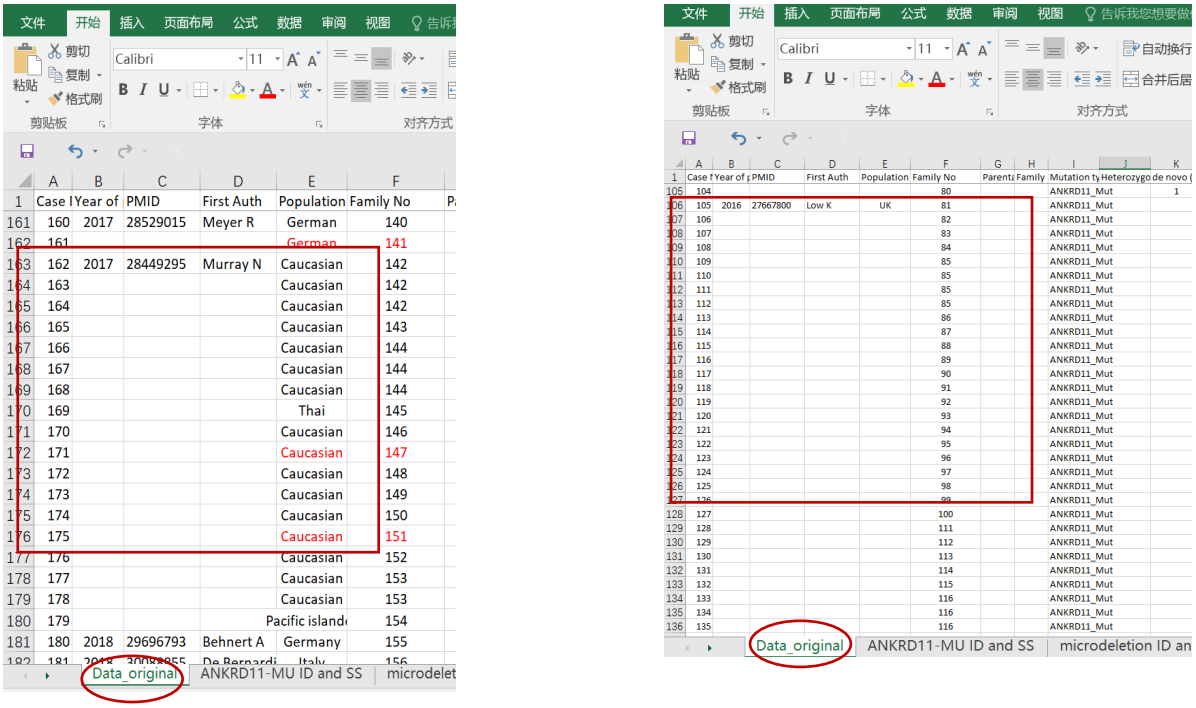


Figure R1 Location of literatures involved in this study in supplementary material

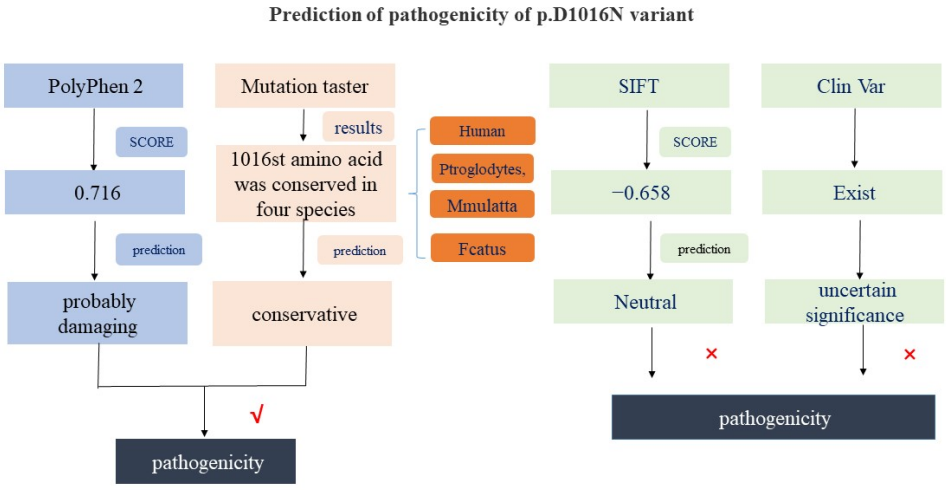
Comment 2: The vast majority of missense mutations in ANKRD11 do NOT cause KBGS. The authors have stated they do not know if this variant is de novo because father is unavailable to test. Photos are not included but they describe ptosis which is not typical for KBGS. In addition

they state the child is of normal intelligence. In fact in contrary to the authors statement pathogenic missense mutations are rarely found in KBGS but are normally associated with at least mild ID. I am very sceptical about normal intelligence in association with a missense mutation in ANKRD11 given how common these variants are in the normal population. The authors need to do further studies to confirm pathogenicity of this variant before claiming this child has KBGS.

Reply 2: Thank you for your comments.

Evidence 1: We understand it would be better if his father's DNA included. Patient 3 were diagnosed KBG syndrome by MDT (Multi Disciplinary Team) in Children's National Medical Center. The variant G3046A in *ANKRD11* was identified by WES analysis and has carefully exclude other candidate genes, and confirmed by Sanger sequencing. Unfortunately, due to some irresistible factors, father's DNA was unavailable. However, we know his father was short stature (-2.1SDS) by the examination could partially support the diagnosis.

Evidence 2: The pathogenicity of p.D1016N variant identified in patient 3 was predicted by several in silico prediction programs as following processes.



Evidence 3: KBGS is a rare genetic disease and has been rarely reported in East Asian. we have merely collected 4 cases with missense mutations until August 2019, and they did not present intelligence disability (see the supporting judgment in supplementary material), however, supporting evidence worth noting that three other cases carrying missense variants reported by other Chinese investigators showed atypical phenotype without intelligence disability (1, 2). The atypical relationship between genotype and phenotype can also be seen in other genes, such as ACAN gene, initial report showed advanced bone age and later more evidences showed

normal even delayed bone age. Thus, we hope in the future there will be more cases could be detected to enrich the phenotypes.

Comment 3: A diagram needs to be included showing the domains and stating location with reference to the D-Box which is the domain that is most frequently discussed in terms of pathogenicity in ANKRD11 mutations.

Reply 3: We thank the reviewer to put forward this important suggestion. We have added the D-box domain (See Figure 2).

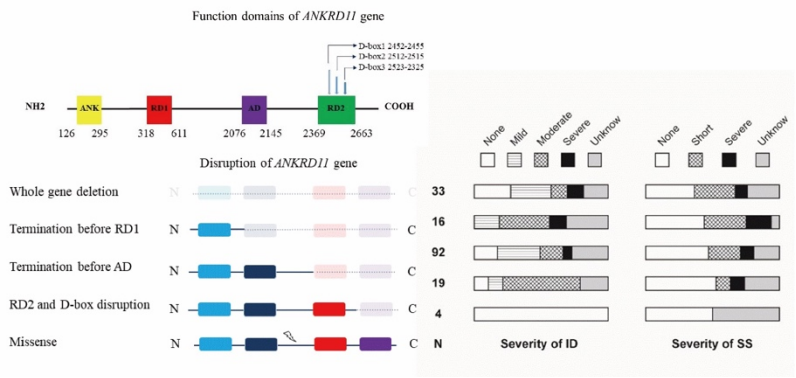


Figure 2 Severity of ID and SS in ANKRD11 gene mutation. The structure diagram of *ANKRD11* gene. It contains 2663 residues and 4 functional domains (ANK: Ankyrin domains, RD1: Repression domain-1, AD: Activation domain, RD2: Repression domain-2). Graphic representation of position of mutation in ANKRD11 gene and the corresponding population (number in the same column as 'N'). The full line shows the retained part, whereas the dash parts showed the truncated part. Decimals represent the total number of groups divided by phenotypes of varying degrees.

Comment 4: Analysis of features with respect to the total number of cases needs to be made clearer. Also how was this done as not all cases will have all features reported. Did the authors rate the photos themselves? All of this needs to be much clearer before you can start stating p values. In particular it is critical that this needs to be clear regarding the heart anomalies as, if true, this could influence change clinical practice in mutation patients.

Reply 4: Thank you for your comments.

This study was completed under the guidance of three professors from MDT, department of Epidemiology, and Department of Endocrinology Genetics and Metabolism of Fudan University. Preliminary screening was conducted on the data and the included images, and incomplete data were deleted. In addition, the process of collection, interpretation and statistical analysis of data were carried out by three researchers in our research group, thus we think our statistic calculation is reliable.

KBG syndrome is a rare disease, with few cases included in each single center, and the clinical phenotypes reported are also different. In order to find regularities among many different phenotypes, more clinical cases in multi-centers need to be included, which is also the direction of the extension of the follow-up research.

Comment 5: Again more clarity needs to be stated regarding how the authors rated ID as there is so much variability in description in the literature. All of this variability should be discussed in the discussions section and it should be clear that this could be leading to biased numbers

Reply 5: We are very grateful to your suggestions. This paper is a kind of secondary processing of the report literature, so it is limited in dealing with the published data. There are various methods to evaluate intelligence, so it is difficult to achieve absolute consistency. In order to ensure the consistency and authenticity of the data from reported literature, the degree of ID was determined based on ① the description of intelligence estimation in each report (e.g. he/she has normal mild/moderate/severe ID, and we rate the corresponding patient as mild/moderate/severe ID. Please see supplementary material "supporting judgment". For your convenience, Figure R2 showed the location of supporting judgement for ID and SS in supplementary material). ② IQ (>3 years of age) in each report, then we rated ID according to DSM-IV stages (i.e. mild: IQ 50-69; moderate: IQ 36-49; severe: IQ 20-35). ③ psychomotor development (<3 years of age infants and young children), then we convert it to DQ (Developmental Quotient). We also rate DQ according to DSM-IV stages (i.e. mild: DQ 50-69; moderate: DQ 36-49; severe: DQ 20-35).

As to your suggestion, we add the following information to the discussion section (see discussion section marked with red color in paragraph 5).

"Our findings showed that patients with *ANKRD11* gene variants disrupting RD1 and RD2 or RD2 alone are more likely to have severe ID, in view of their higher risks, absolute benefits from early recognition of KBG syndrome can be achieved. Although we detected significant differences between the effect of different domain in ANKRD11 protein on intelligence, we cannot exclude the possibility that these effects might affected by methodological differences for evaluating ID".

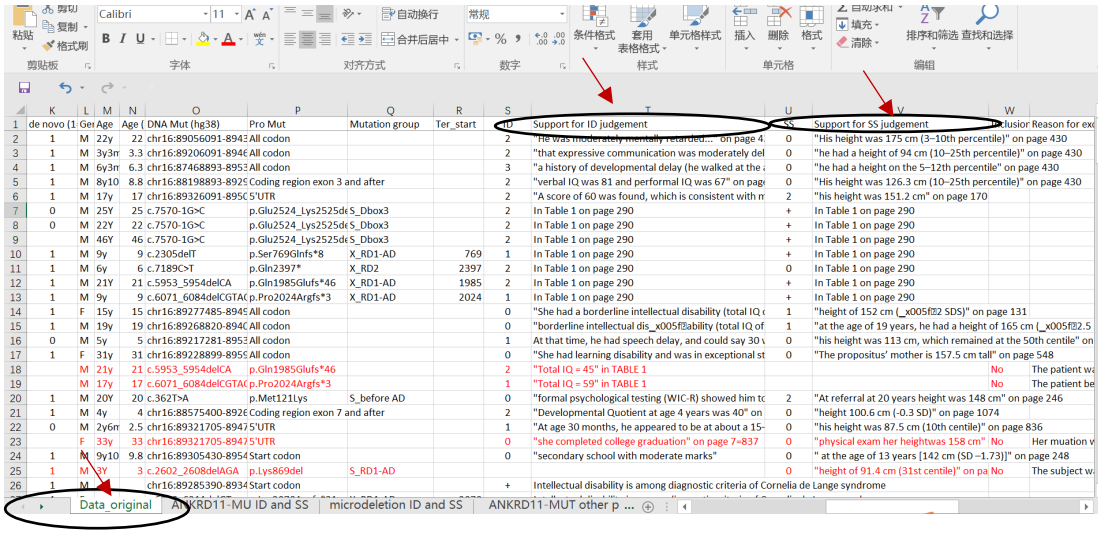


Figure-R2 Location of supporting judgement for short stature and intelligence disability in supplementary material

Comment 6: There are a significant number of spelling and English language errors which need to be addressed.

Reply 6: Following your advice, we have modified this paper repeatedly to reduce the misspellings, the grammar mistakes and the inaccurate description as far as possible. This has been done by the editing company as well.